A Study of the Blood of certain Coleoptera: Dytiscus marginalis and Hydrophilus piceus.

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With Plate 11.

Synopsis of Contents.

Introduction.

THE present investigation had its origin in a study of the cell changes occurring in malignant growths, during the course of which attention was directed towards the presence of wandering cells in such growths. It appeared likely that light would be thrown upon the morphology and life-history of the wandering cells of the higher vertebrates by comparison with the free cells of the blood of various invertebrates. To this end the present investigation, which is confined to Coleoptera, was undertaken. The work, however, extended beyond the limits originally assigned, for it became necessary,

partly in order to prepare an isotonic fluid for the blood-cells studied, and partly in order to determine the nature of the medium in which they lived, to examine the fluid part of the blood also.

The literature of the subject is scattered and appears to be very scanty, so that further research in the light of the more recent development of methods of investigation seemed very desirable.

As early as 1864 Landois (1) studied the blood of insects, noting the colour, smell, and reaction, and ascertaining the presence of iron in the serum. He did not, however, give a definite classification of the blood-cells, though he states that division takes place by the nucleus usually splitting into two parts.

The morphology of the formed elements in Molluscs and Arthropods was further studied by Cattaneo (2, 1889) and Wagner (3, 1890).

Cuénot (4, 1891) gave a voluminous but not very illuminating contribution to the literature of the blood of invertebrates. This author observed that the blood of Hydrophilus piceus is at first pale yellow, and when exposed to the air becomes altered resembling caramel; neither uranidin, lutein nor fibrin is present; the albuminoid present, which coagulates at 60°–61°, is called hæmopheine. The blood of Blaps, which is also pale yellow, and on oxidation becomes quickly ochreous yellow, contains an albuminoid which is regarded as identical with hæmopheine.

An important observation in respect of the Coleoptera was made by Durham (5, 1892), who ascertained that the blood-cells of Dytiscus exhibited phagocytosis, readily ingesting particles of Indian ink.

Reference may here be made to a much more exhaustive examination of the cœlomic fluid of Lumbricus by Lim Boon Keng (6, 1896). This author found that the cœlomic fluid had a specific gravity of 1·007 to 1·009, and was of alkaline reaction; it also contained crystals, pigment and microbes, and held cells in suspension, some of which exhibited phagocy-

tosis. The latter were divided into—small non-granular, large hyaline, small granular, large granular and chloragogen cells, and also spindle cells.

Some interesting observations were made by Benham (7, 1901) on the cœlomic fluid of Acanthodrilids, which was found to undergo a sort of coagulation on standing, becoming white, sticky, and slimy. The cellular elements of the cœlomic fluid are divisible according to Benham into four groups ; amœbocytes (granular cells), eleocytes (containing fatty globules), lamprocytes (containing granules), and linocytes (containing threads).

Hollande (8, 1909) divides the cellular elements of Coleoptera into three groups : lymphocytes, granular leucocytes, and leucocytes with spherules.

The Coleoptera selected for the present investigation have been Hydrophilus piceus (Linn.) and Dytiscus margin. alis (Linn.).

Mode of Collecting Blood.

In order to obtain blood from Hydrophilus and Dytiscus the following procedure was adopted. The wing cases were lifted up and pinned aside in a paraffin dish. The wings were then divided with scissors, so as to display the dorsal segments of the abdomen. One of the dorsal segments was next opened at the side and a flap of chitin cut off after being previously freed from adherent connective tissue. The blood which was seen lying in the body cavity between the viscera was then removed drop by drop by means of a capillary tube. If this is carefully done it should be possible to withdraw blood without damaging any organ or setting free any cells derived from the body tissues.

General Characters of the Blood of Dytiscus and Hydrophilus.

The average amount of blood obtainable from Hydrophilus piceus was ·32 c.c. The amount of blood obtained

from five specimens (in April) was found to measure 1·6 c.c. (average amount ·32 c.c. from each) ; later in the same month three specimens yielded ·43 c.c. (average amount ·14 c.c. from each) ; on another occasion (in July) ·26 c.c. per specimen was obtained.

From Dytiscus the average amount obtainable was ·10 c.c. As affording some idea of the range observable the following data may be given : ·42 c.c. obtained from three specimens in February (average amount ·14 c.c. from each) ; ·375 c.c. obtained from six specimens also in February (average amount ·063 c.c. from each) ; 1·3 c.c. obtained from twelve specimens in March (average amount ·108 c.c. from each) ; 1·65 c.c. obtained from seventeen specimens in April (average amount ·10 c.c. from each).

The blood was found on centrifugalisation to consist partly of fluid and partly of suspended material. The latter was variable in different animals, but was relatively small both in Hydrophilus and in Dytiscus, amounting in the observations made to about 1 per cent. (by volume) of the blood.

The suspended material consisted partly of cells, partly of free granules. The latter are described in detail below in connection with the blood-plasma ; the former are taken in the succeeding section. The cells formed a relatively small amount of the precipitate obtained on centrifugalisation, but owing to the circumstance that the two constituents of the precipitate cannot be separated, no quantitative comparison of the two could be made.

Characters of the Blood-Plasma of Dytiscus Marginalis.

Colour and Spectroscopic Appearance.—The blood-plasma immediately after removal was, in a layer four millimetres thick, of a deep amber colour, subsequently changing at the surface of contact with the air to dark brown, almost black (well seen when the blood was kept in a narrow pipette, the upper layer of liquid becoming deeply coloured, while that below, where access of air was prevented, remained

unchanged). Since the blood darkened on exposure to air, or rather to oxygen, it follows that it contained exceedingly little dissolved oxygen in the straw-yellow condition which it exhibited in the living body. On spectroscopic examination of a layer six millimetres thick the portion of the spectrum lying to the blue end of the green was completely cut off and the green itself in part absorbed, but the red of the spectrum was little altered. When the blood had become darkened the spectrum became dim but no absorption bands were seen. The brownish-black colour which the blood assumed on exposure to air could not be removed by adding ammonium sulphide.

Odour.—The blood immediately after collection had a sweet smell somewhat resembling malt extract, but was also distinctly offensive. A faint odour of free ammonia was recognisable. On adding sodium hydrate and boiling, the issuing vapour readily turned neutral litmus paper blue, thus affording additional evidence of the presence of ammonia or an ammonium salt (it will be seen below that carbon dioxide was present in the blood-plasma).

Specific Gravity.—This ranged, in the specimens examined, from 1·025 to 1·027.

Reaction.—The blood when examined immediately after collection was always found to be alkaline to litmus paper.

Basicity and Acidity.—Observations were made immediately after removal of the blood from beneath thoracic or abdominal tergites, great care being taken to avoid injury to viscera. In every case it was found that the blood, which was strongly alkaline to litmus on removal, still remained alkaline on adding an equal volume of $\frac{N}{30}$ HCl; on adding an equal volume of $\frac{N}{20}$ HCl it became faintly alkaline; on adding an equal volume of $\frac{N}{10}$ HCl it became acid to litmus paper. The basicity of the blood-plasma (which is in part due to ammonium carbonate) is therefore slightly greater than is

represented by a $\dfrac{N}{20}$ solution of hydrochloric acid. As the blood-plasma is alkaline to litmus its basicity cannot be determined by adding potassium hydrate.

Coagulation.—No spontaneous coagulation of the blood of Dytiscus marginalis occurred on standing.

Composition.—The blood-plasma was found to contain 6·6 per cent. to 10·4 per cent. of solids, dried at 110° C. (1 c.c. of blood was taken for estimation of total solids).

On rendering the blood slightly acid with acetic acid and then heating, it became solid. When the blood was diluted with three parts of distilled water or ·85 per cent. solution of sodium chloride, made slightly acid with a 1 per cent. solution of acetic acid and boiled, a brown precipitate formed. After centrifugalisation the supernatant liquid was found to remain turbid (this being apparently due to the presence of ammonium salt in the plasma), so that complete separation of the proteid from the non-proteid solids of the plasma could not by this means be effected. By weighing the brown precipitate after drying at 110° C. it was found that the former was not less than 1·3 per cent.; this figure has little value, however, since the supernatant liquid contained proteid forming a gelatinous mass as evaporation proceeded.

On adding blood to a large excess of distilled water turbidity appeared, followed by the formation of a white precipitate, showing the presence of globulin.

The dried solids of the plasma contained about 9 per cent. of ash, which was of a brownish-white earthy aspect. Owing to the small amount available further analysis of the ash was not possible.

Osmotic Pressure.—The freezing-point of the blood-plasma determined by Beckmann's method (1·3 c.c. of blood-plasma were employed) was − ·77° C., corresponding to an undissociated $\dfrac{M}{23}$ solution.

Granular Material.—This consisted of granules 1 μ to ·2 μ in diameter, exhibiting Brownian movement and in part

precipitated on centrifugalisation. In addition numerous ultra-microscopic particles of much smaller size were recognisable on strong illumination against a dark background. The former granules when a film of blood was prepared by Leishman's method (alcohol fixation, staining with methylene-blue-eosin) stained blue.

Gases Dissolved in Blood.—1·65 c.c. of blood-plasma (obtained from seventeen Dytisci) were placed in connection with a Toepler pump and ·14 c.c. of gases extracted. On exposing this to the action of a 10 per cent. solution of caustic potash the volume was reduced by ·11 c.c. On adding a 50 per cent. solution of caustic potash containing 2·3 per cent. of pyrogallol a very slight diminution of volume, too small to determine, occurred, and ·03 c.c. of gas remained behind, representing nitrogen and argon. The percentage amounts of dissolved gases were therefore :

Carbon dioxide	.	.	.	.	6·7 per cent.
Nitrogen	.	.	.	.	1·8 ,,
					——
Total	.	.	.	.	8·5 ,,

Characters of the Blood-Plasma of Hydrophilus Piceus.

Colour and Spectroscopic Appearance.—Immediately after collection the blood was, in a layer four millimetres thick, of a straw-yellow colour. Subsequently it became dark brown, the change first appearing at the upper surface, in contact with the air. When kept in hydrogen the blood remained for several hours of a pale yellow colour, whence it follows that the darkening is due to absorption of oxygen. The tension of dissolved oxygen in the blood must, therefore, be very low. On spectroscopic examination of the blood in a layer 18 mm. thick a general darkening of the spectrum was observed, the extent of the spectrum diminishing towards both the red and the blue, but no absorption bands were visible. When darkening of the blood occurred on standing

still further obscuration of the spectrum took place, but no absorption bands appeared.

Odour.—The blood had a faint offensive odour resembling decaying grass. No distinct odour of free ammonia could be detected, but on adding the blood (collected from the living insect a few minutes before use) to a solution of caustic potash (previously ascertained to be free from ammonia) and boiling, the issuing steam readily turned neutral litmus paper blue, showing the presence of an ammonium salt.

Specific Gravity.—This was found to be 1·012 (only one specimen was examined).

Reaction.—The blood examined immediately after collection was alkaline to litmus paper.

Basicity and Acidity.—The blood was tested immediately after collection, great care being taken to avoid injury to viscera during collection. The reaction remained slightly alkaline to litmus when mixed with an equal volume of $\frac{N}{50}$ HCl; when an equal volume of $\frac{N}{40}$ HCl was added the reaction became neutral to litmus; when an equal volume of $\frac{N}{30}$ HCl was added the reaction became acid. The basicity of the blood-plasma is therefore represented by a $\frac{N}{40}$ solution of hydrochloric acid. Since the blood had an alkaline reaction its acidity could not be determined by the addition of caustic potash. It is obvious that the basicity was, as in the case of the blood-plasma of Dytiscus, in part due to the presence of ammonium carbonate, already referred to.

Coagulation.—No spontaneous coagulation of the blood occurred on standing.

Composition.—The blood-plasma contained 11·6 per cent. of solid matter (·43 c.c. of plasma was taken for the estimation of total solids).

The plasma contained proteid coagulable on acidifying with acetic acid and boiling, but as was the case with that of

Dytiscus, complete precipitation did not occur, so that a quantitative estimation of the amount of coagulable proteid was not possible.

On diluting the blood with ten times its volume of distilled water a copious white precipitate formed, showing the presence of globulin.

The dried solids of the plasma contained about 3 per cent. of ash of a white, porous, earthy aspect.   Owing to the small amount of ash obtainable no determination of its composition could be made.

Osmotic Pressure.—The freezing-point of the blood-plasma, determined by Beckmann's method (1 c.c. of fluid was employed), was −·647° C., corresponding to an undisso-ciated $\frac{M}{28}$ solution.

Granular Material.—This consisted of small particles, exhibiting Brownian movement, ·2 μ to 2 μ, in diameter, the former being the more numerous.   In addition ultra-microscopic particles less than ·2 μ in diameter could be seen on strong illumination on a dark background.   The granules increased in number on standing; some of the larger granules may have been derived from the disintegration of the blood-cells.   The granules, in films fixed by Flemming's solution, stained by basic dyes.

Gases Dissolved in Blood-plasma.—By means of a Toepler pump the dissolved gases contained in 1·6 c.c. of blood-plasma (obtained from five Hydrophili) were collected and were found to measure ·09 c.c.   After the absorption of carbon dioxide by caustic potash the volume of gas was reduced to ·03 c.c.   Very little further reduction could be obtained by the action of pyrogallol in strongly alkaline solution.   The percentage of dissolved gases was therefore :

Carbon dioxide   .   .   .   .   3·8 per cent.
Nitrogen   .   .   .   .   .   1·9   ,,
                                                ——
    Total   .   .   .   .   5·7   ,,

It will be noticed that no dissolved or loosely combined oxygen was obtained from the blood-plasma of Dytiscus and Hydrophilus. When oxygen was absorbed in vitro the blood-plasma became darkly coloured. It follows, therefore, that as long as the blood-plasma remains straw-yellow coloured the absence of dissolved oxygen may be inferred. No data are, however, available to indicate the means by which darkening of the circulating fluid is avoided in the living insect. The blood appears to serve solely as a nutritive medium. The tissue-cells, it may be observed, are in direct relationship to the finest ramifications of the tracheal vessels (**9**), which penetrate to all parts of the body of these insects. From the tracheal vessels the tissue-cells appear to derive their supply of oxygen directly, not being dependent on the mediation of the blood-plasma as in mammals and in animals living exclusively in water.

The Characters of the Blood-cells.

The blood-cells were studied in films fixed in Flemming's strong solution, without previous drying, and also in dry films. In addition, Flemming's solution was added to the blood, and the formed elements, after centrifugalisation, embedded and cut in paraffin.

The stains chiefly used were Heidenhain's iron-alum hæmatoxylin, Breinl's methylenblue-saffranin-orange G. triple stain and basic fuchsin-methylenblue-orange G. triple stain. Intra vitam methylenblue staining was also employed.

In Dytiscus marginalis and Hydrophilus piceus the blood consists of flocculent suspended material made up of fine granules, about 1μ to $\cdot 2 \mu$ in diameter, and of cells. These latter are of two kinds—(1) phagocytes, and (2) small round-cells.[1]   The number of cells counted varied from 120 to 500 per cubic millimetre in Dytiscus, and from 1030 to 4440 per cubic millimetre in Hydrophilus.

The phagocytic cells are usually spindle-shaped when seen

[1] We have not observed blood-platelets in the plasma.

on edge, and round, with two polar prolongations, when viewed from above. They measure in both Dytiscus and Hydrophilus from $17\,\mu$ by $19\,\mu$ to $15\,\mu$ by $30\,\mu$. In Dytiscus the cytoplasm of these cells is coarser and more largely vacuolated than in Hydrophilus. The nucleus in Dytiscus has a definite membrane and the chromatin is diffusely and irregularly distributed. Faint strands of linin connect together the chromatin masses. Generally only one nucleolus is present (see figs. 1–4). In Hydrophilus a well-defined nuclear membrane is also present, but otherwise the nucleus is strikingly different in appearance to that of Dytiscus, for instead of being distributed in unequal masses, as in the latter insect, the chromatin occurs in the form of about twenty-five to thirty nearly equal-sized aggregations, and these generally appear to be split in one direction, giving the appearance of twin masses of chromatin.[1] The linin is inconspicuous (see figs. 11–13). When these cells have ingested foreign particles from the plasma they change their shape, gradually drawing in their polar extensions and becoming more or less round (see figs. 6–9 and 12–14). Both in the fresh and well-fixed blood of Dytiscus and Hydrophilus it can be seen that the majority of the phagocytes which contain no food-particles or recent food-vacuoles in their cytoplasm possess the polar prolongations. At all times the phagocytes may exhibit short and thin pseudopodia extruded from various parts of the cytoplasm, but the polar extensions, although of a more permanent nature, are themselves only pseudopodia, and are distinctive of that phase in the life of the cell in which no ingestion and digestion occur.

The other kind of cell found in the blood is a small cell, with large nucleus and very little cytoplasm (see figs. 10 and 18). These cells, for want of a more convenient term, we designate as small round-cells. As in the case of the phagocytes,

[1] This arrangement of the chromatin in twin groups is apparently characteristic of the somatic cells of Hydrophilus. It can be seen, for instance, in the Malpighian tube cells, in the cells of the glands of the mid-gut, and in the spermatogonia.

the cytoplasm of these cells is coarser in Dytiscus than in Hydrophilus. Small round-cells are present in the blood in much smaller number than are phagocytic cells, varying from one in fifty to one in thirty of the total number in Dytiscus, and amounting to one in fifty or less in Hydrophilus.

In the phagocytic cells, a series of interesting changes follow the ingestion of solid particles, which may now be described in some detail.

In Dytiscus the ingestive activity of these cells is very great. Thus, if a solution of Indian ink be injected into the abdominal cavity, it can be seen that after a few hours most of the phagocytes have particles of the ink in their cytoplasm, as is illustrated by fig. 4 (four and a half hours after injection). As digestion proceeds a clear area appears round each particle, becoming a well-defined vacuole later on. (see figs. 4, 6 and 8). A part of the ingested matter is not digested, being eventually ejected into the plasma. The vacuoles slowly contract until they become indistinguishable in the schaumplasma. The nucleus undergoes the following changes during the digestion of ingested matter. The chromatin becomes more plentiful (cf. figs. 6 and 7 with 1 and 4) ; the nuclear membrane invaginates here and there and eventually the nucleus becomes multilobulate (see fig. 8), some cells being even polynuclear (see fig. 9).

In Hydrophilus the changes which take place in the cytoplasm after the ingestion of solid particles are similar to those of Dytiscus. The nucleus undergoes only a slight change, the chromatin becoming more diffuse and the linin strands more apparent (cf. fig. 12 with figs. 13 and 14). Lobulation of the nucleus does not occur. As in Dytiscus some of the granules, representing probably indigestible portions of the ingesta, are extruded from the cytoplasm and discharged into the surrounding blood-plasma. The staining reaction of the solid ingesta changes, for in the cytoplasm at first these take the acid stain, later they are stained by a mixture of the acid and basic stains, and ultimately, at the

time of extrusion, the undigested residue becomes increasingly basic in its staining reaction.

On examining with dark background illumination a drop of blood immediately after removal, both forms of cells, phagocytes and small round cells, which are observed to be very granular, are seen to exhibit very numerous fine pseudopodia, about $\cdot 2\,\mu$ in diameter and of length occasionally surpassing that of the cell. No amœboid movements are seen, but after a time the granules of the protoplasm exhibit Brownian movement, which ultimately ceases when death has occurred, without the cell, however, becoming vacuolated as do human leucocytes. At the time of death the pseudopodia have become somewhat indistinct, their situation being indicated by granules apparently derived from the protoplasm.

The mode of division of the phagocytes does not always appear to be the same. In Dytiscus only amitotic division was seen (fig. 5), and that comparatively rarely. In Hydrophilus, however, mitotic divisions were fairly common (see figs. 15 and 16), but no amitotic forms were met with. It is quite possible, however, that both forms of division occur in the two genera examined, but we have nevertheless been unable to observe either mitotic division in Dytiscus, or amitotic division in Hydrophilus. We have not been able to observe cell-division in the small round-cells.[1]

The origin of the free cells in the blood of insects has been attributed to various sources by different writers. Cuénot (10) and Balbiani (10) derive the leucocytes from the pericardial cells. Schäffer (10) derives at least some of these cells from the fat-body cells, but Kowalewsky (10) denies any leucocyte formation to either the pericardial or fat-body cells, asserting that the blood-cells arise from special nests or islands of tissue near the heart.

[1] Hollande (loc. cit.), in dealing with other Coleoptera, Coccinella. Mysia and Epilachnia, describes phagocytes similar to, but considerably smaller than, those above described. These reproduce by mitosis. The other class of cells which he described in those species, viz. the " cellules à sphérules," is entirely absent in Dytiscus and Hydrophilus.

Our own observations do not permit us to make any confident statement as to the origin of the blood-cells from any of the tissues of the body. It appears to us, however, that the evidence adduced in favour of the above suggested modes of origin is altogether inadequate, for both the pericardial and fat-body cells are totally different in aspect from the blood-cells. Thus the pericardial cells of Dytiscus and Hydrophilus are considerably larger than the largest blood-cell, and their nuclei are relatively smaller, while their cytoplasm is very abundant. Again, if the blood-cells arose from any of the above sources, transitional forms would be present; these we have failed to observe.

On the other hand, whatever other mode of origin of the blood-cells may exist, it is clear, since division figures occur in Hydrophilus and Dytiscus (mitotic and amitotic), that the supply of these cells is kept up, in part at any rate, by multiplication in the blood-plasma.

COMPARISON WITH MAMMALIAN BLOOD.

In the above investigation we have applied the term "blood" to designate the circulating fluid of Coleoptera. This fluid presents, however, several important points of difference from the blood of the higher mammalia. It will, therefore, be of advantage briefly to contrast the characters of these two fluids so far as the present limitations of knowledge permit a comparison to be made.

The blood of Dytiscus and Hydrophilus resembles mammalian blood in so far as it consists of an albuminous fluid containing cells. The fluid part resembles mammalian blood-plasma in containing proteid, coagulable by heat. Whether more than one form of heat-coagulable proteid is present cannot as yet be stated, but it may be observed that a globulin precipitable by dilution with distilled water, and therefore held in solution by the saline constituents of the fluid, is also present as in mammalian blood. The cellular elements of mammalian blood are represented in the blood of Dytiscus

and Hydrophilus by phagocytes and small round-cells. In the phagocytes of Dytiscus fragmentation of the nucleus occurs, which is comparable to that seen in polynuclear leucocytes.

Turning now to points of difference, the low osmotic pressure of the blood of Dytiscus and Hydrophilus, represented by a $\frac{M}{23}$ to $\frac{M}{28}$ (undissociated) solution, contrasts with that of mammalian blood, represented by a $\frac{M}{7}$ (dissociated) solution. A more striking point of difference is the absence of cells containing hæmoglobin in the blood of the former, which is also free from dissolved hæmoglobin. Moreover, the white cells in Dytiscus and Hydrophilus are scanty, relatively to those of mammalian blood; no oxyphile granules can be recognised in the phagocytes when fixed and stained by Leishman's method, nor are platelets present. Mitoses are not uncommon in the phagocytic cells of Hydrophilus. In both Dytiscus and Hydrophilus no spontaneous coagulation of the blood occurs on standing. In both insects oxygenation appears to occur by direct transfer to the tissues of oxygen supplied by the tracheal vessels, the blood-plasma not serving as a medium of exchange.

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10. Quoted by Packard, loc. cit., pp. 419–423.

EXPLANATION OF PLATE 11,

Illustrating the paper by Dr. J. O. Wakelin Barratt and Mr. George Arnold entitled "A Study of the Blood of certain Coleoptera: D y t i s c u s m a r g i n a l i s and H y d r o p h i l u s p i c e n s."

[The drawings are made using a 2 mm. apochromatic oil-immersion objective with 8 and 18 compensating oculars, are drawn without the aid of a projection apparatus, and are represented as stained by methylene-blue and orange G. Magnification 1250 diameters.

Figs. 1–9.— D y t i s c u s.   Phagocytic cells are represented in figs. 1–8; small round-cells in fig. 10.

Figs. 1–3.—Phagocytic cells in the active condition with pseudopodia. Figs. 1 and 3 viewed from above; fig. 2 seen on edge.   Nucleus entire, chromatin inconspicuous.   Cytoplasm contains no food-vacuoles in figs. 1 and 3.

Fig. 4.—Phagocytic cell with recently ingested particles of Indian ink in the cytoplasm. Vacuoles have not yet formed round the particles.

Fig. 5.—Phagocytic cell exhibiting amitotic division, nearly completed.

Figs. 6 and 7.— Phagocytic cells exhibiting active digestion.   In fig. 6 the ingested matter is in close contact with, and depressing, the

nucleus; the remains of a food-vacuole are seen below. In fig. 7 the process is more advanced: vacuoles surround two of the ingested particles, which are being digested.

Fig. 8.—Phagocyte after digestion. Vacuoles empty. The nucleus has become lobulate.

Fig. 9.—Phagocyte in the resting condition, showing numerous small pseudopodia. Nucleus fragmented.

Fig. 10.—Small round-cells. The characteristic feature of these cells is the small amount of cytoplasm, which forms a thin layer round the nucleus.

Figs. 11–18.—Hydrophilus. Phagocytic cells are represented in figs. 11–17 ; small round-cells in fig. 18.

Fig. 11.—Phagocytic cell, with pseudopodia, seen on edge. A small particle surrounded by a vacuole lies in one pole.

Figs. 12–14.—Phagocytic cells with ingested particles. After a time these particles become enclosed in vacuoles, and, as digestion proceeds, lose their staining reaction.

Fig. 15.—Phagocytic cell. Polar view of mitotic division-figure.

Fig. 16.—Phagocytic cell. Equatorial view of mitotic division-figures.

Fig. 17.—Large phagocyte with abundant cytoplasm containing numerous flaky granules.

Fig. 18.—Small round-cells. The characteristic feature of these cells is the scanty cytoplasm surrounding the nucleus.

nu. Nucleolus. f. Ingested material.